

The clinical value of serum transferrin receptor measurements

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INTRODUCTION

The dominating mechanism for iron delivery to tissues is the internalisation of the transferrin receptor-diferric transferrin complex. Other ways for iron to enter cells exist but do not contribute significantly to the cellular iron homeostasis in humans. Once the complex has been endocytosed, iron is released to the cytosol for further transport inside the cell, for example to the mitochondria. The receptor-apotransferrin complex recycles to the cell surface for a renewed round of iron uptake. A fraction of the transferrin receptors are cleaved inside the endocytotic vesicle and finally shed into the blood as truncated transferrin receptor monomers complexed with apotransferrin. The serum concentrations of these so called serum transferrin receptors (sTfR) correlate with erythropoietic activity and tissue iron demands. This review will focus on the clinical value of sTfR measurements.

TRANSFERRIN RECEPTORS

Two different receptors for transferrin are known: transferrin receptor 1 and the newly discovered transferrin receptor 2. In this review transferrin receptor (TfR) alludes to transferrin receptor 1.

The transferrin receptor is a type II transmembrane protein. It is a homo-dimer consisting of two identical monomers joined by two disulfide bindings at cysteine residues 89 and 92 in the extracellular domain just outside the cell membrane (Jing and Trowbridge 1987). The monomer is a glycoprotein with molecular mass 90 kDa consisting of 780 amino residues. The protein has three domains: one 61-residue amino-terminal cytoplasmic region, a 28-residue transmembrane region and a 671-residue extracellular carboxyl-terminus (McClelland et al 1984, Schneider et al 1984). Each ectodomain can bind one molecule of transferrin and thus the transferrin receptor can bind two molecules of transferrin carrying in total four Fe^{3+} . The ectodomain consists of three regions, each contributing to and critical for the transferrin binding (Lawrence et al 1999). The structure of the ectodomain has striking similarities to the membranebound carboxy peptidase II (Lawrence et al 1999), suggesting that they have evolved from a common peptidase (Bzdega et al 1997). The transferrin receptor however has no peptidase activity (Lawrence et al 1999, Bzdega et al 1997).

The transferrin receptor is glycosylated posttranslationally. One O-linked and three N-linked glycosylation sites are positioned on the extracellular domain (Omary and Trowbridge 1981). Receptors without the O-linked glycosylation are cleaved inside the cell with release of the extracellular domain. Mutations at the N-linked positions confers inability to bind to transferrin (Rutledge and Enns 1996, Williams and Enns 1991). Through acetylation with palmitate the transmembrane domain is also modified posttranslationally. (Omary and Trowbridge 1981, Schneider et al 1984). The cytoplasmic domain is necessary for clustering of the receptor-transferrin complex leading to the formation of coated pits in the cell membrane and subsequent endocytosis (Iacopetta et al 1988, Rothenberger et al 1987). Effective internalisation of the complex is initiated by phosphorylation and dephosphorylation in a conserved signal sequence, YTRF, in the cytoplasmic domain (Collawn et al 1990, 1993).

Almost all animal cells express the transferrin receptor, with the exception of terminally differentiated cells such as mature erythrocytes. In particular, the receptor is expressed, 10 000 to 100 000 molecules per cell, on the surface of proliferating cells (Gatter et al 1983, Chitambar et al 1983). Non proliferating cells have a very low or absent expression of the receptor. It has been suggested that the coupling between cell proliferation and expression of the transferrin receptor is mediated by the enzyme ribonucleotide reductase. Epithelial cells in kidneys, cervix, breast, testicles, esophagus, stomach, endocrine pancreas, hepatocytes and the pituitary gland have a basal expression of the transferrin receptor. Besides vividly proliferating cells a high expression of the transferrin receptor is found on immature erythrocytes, placental syncytiotrophoblasts and in the liver (Iacopetta et al 1982, Loh et al 1980). The transport of iron into the brain is regulated by the expression of the transferrin receptor on capillary endothelial cells and on epithelial cells in the choroid plexus. Neurons also express the transferrin receptor.

The gene for the human transferrin receptor is 32 Kb long and located on chromosome 3. It shows some similarities to the gene for prostatic specific antigen. On chromosome 3 also reside the genes for transferrin and the transferrin like membrane bound paratransferrin. The transferrin receptor gene is transcribed into a 5 Kb mRNA with a large 3' region that is not translated. 100 base pairs upstream from the starting point for transcription lies the promoter region neces-

sary for basal and activated transcription in proliferating non-erythroid cells. In this region the hypoxia responsive element is found among others.

In non erythroid cells the expression of the transferrin receptor is regulated by posttranscriptional stabilisation or degradation of mRNA. In the non-translated 3' region there are five iron responsive elements (IRE) to which iron regulatory proteins (IRP) bind when intracellular iron concentration is low. Bound IRP blocks the cleavage site from an endonuclease and stabilises mRNA thereby prolonging the half time from 30 minutes to six hours. The synthesis of ferritin and in some extension of transferrin is also regulated by the same IRPs. When they bind to a 5' IRE on ferritin mRNA translation is blocked and the synthesis of ferritin is diminished.

In erythroid cells the expression of transferrin receptor is regulated not by the intracellular iron concentration but by upregulated transcription during the differentiation of the erythroid cells.

The transferrin receptor binds diferric transferrin with high affinity but not apotransferrin at the pH found in blood. In the endosomal acidic milieu iron is released. The hemochromatosis gene product associates with the transferrin receptor and B2-microglobulin at the cell surface and remains associated with the receptor during the endo/exocytotic cycle (Gross et al 1998). HFE participates in the control of cellular iron uptake and intracellular iron delivery; in the former case by decreasing the receptor's affinity for transferrin and abrogating transferrin receptor endocytosis (Feder et al 1998, Roy et al 2000, Salter-Cid et al 2000).

A second transferrin receptor (TfR2), predominantly expressed in the liver, has been described (Kawabata et al 1999) and the gene mapped to chromosome 7q22. The extracellular domain of TfR2 has 45% identity and 66% similarity with TfR. TfR2 mediates cellular uptake of iron and this mechanism is not regulated by iron (Fleming et al. 2000). A mutation in the TfR2 gene leading to hemochromatosis type 3 has been identified (Camaschella et al. 2000).

THE SERUM TRANSFERRIN RECEPTOR

A truncated form of the transferrin receptor can be found in serum (Kohgo et al 1986). This was first noted when reticulocytes maturing to erythrocytes were observed to lose their transferrin receptors by shedding them to the blood (Pan et al 1983). This serum transferrin receptor (sTfR), a 74-kDa monomer, is the extracellular domain of the transferrin receptor cleaved at Arg100-Leu101 inside the endosomes (Shih et al 1990, Baynes et al 1993). After exocytosis sTfR bound to transferrin circulates in blood in an amount proportional to cellular TfR (Beguin et al 1988). Elevated levels of sTfR may thus reflect increased erythropoietic activity or mass as seen in thalassemia and hemolytic anemias, whereas decreased levels are seen in for example aplastic anemia.

There is no difference in sTfR values for healthy adult men and women and no correlation of [sTfR] with the age

of the subject (Skikne et al 1990, Flowers et al 1989). Black subjects have significantly higher concentrations than nonblacks, and people living at high altitude have higher concentrations (Huebers et al 1990, Flowers et al 1989, Allen et al 1998, Vernet et al 2000).

The sTfR-levels are higher in children, especially in infants, than in adults decreasing gradually from the neonatal period to adolescence (Anttila et al 1997, Virtanen et al 1999, Yeung et al 1997, Lonnerdal et al 1994, Choi et al 1999, Suominen et al 2001).

No gender differences in sTfR concentration exists for children except for neonates, where males have slightly higher concentrations (Choi et al 2000).

In the pregnant woman sTfR increases with gestational time and returns to normal 5 to 12 weeks after delivery (Choi et al 2001, 2000, Akesson et al 1998), but conflicting results have been reported (Carriaga et al 1991).

sTfR promises to be useful for detecting iron deficiency in pregnancy (Rusia et al 1999, Carriaga et al 1991).

In blood donors sTfR may be slightly elevated (Punnonen et al 1999, Vernet et al 2000, Bolton et al 2000).

The day-to-day intra-individual variation and the overall biological variation in sTfR is low (Cooper et al 1996).

sTfR increases when exogenous erythropoietin is administered. Erythropoietin administration in patients undergoing hemodialysis or in healthy athletes increase sTfR significantly after one week of drug administration with a maximum 30-40 days later (Lorenzo et al 2001, Beguin et al 1995, Birkeland et al 2000, Parisotto et al 2000).

SERUM TRANSFERRIN RECEPTOR IN DISEASE

Iron-deficiency anemia is associated with high concentrations of transferrin receptor in serum reflecting the degree of iron depletion. (Punnonen et al 1994, 1997, Suominen et al 1997, Chua et al 1999).

There is no advantage in using sTfR instead of other traditional parameters for detecting iron deficiency in uncomplicated anemia or as sole discriminator in unselected anemia (North et al 1997, Beguin et al 1993).

In non-anemic persons with functional iron deficiency, commonly seen in pregnancy, women of childbearing age, small children, adolescents and blood donors, sTfR is modestly increased and decreases with iron supplementation (Suominen et al 1998, Anttila et al 1997, Zhu et al 1998). It has been questioned if sTfR or the sTfR-ferritin ratio really contributes to the identification of sub clinical iron deficiency (Gimferrer et al 1997).

sTfR has been evaluated in masked iron deficiency in chronic renal failure patients. In patients on regular hemodialysis but not treated with erythropoietin sTfR is higher among those with iron deficiency than among those that are iron replete (Ahluwalia et al 1997, Fernandez-Rodriguez 1999). The baseline sTfR before start of erythropoietin or the initial response in sTfR to an increased erythropoietin dose can predict a hemoglobin response in patients already on erythropoietin (Beguin et al 1993, Ahluwalia et al 1997). sTfR cannot reliably detect masked iron

deficiency in anemic chronic hemodialysis patients on maintenance erythropoietin because of increased erythropoiesis, which itself raises serum TfR levels (Ahluwalia et al 1997, Hou et al 1998, Fernandez-Rodriguez 1999).

In chronic diseases serum ferritin is often increased irrespective of iron status and can thus not be used as a marker for iron deficiency. sTfR or sTfR-ferritin ratio have been shown to be of diagnostic value for detecting iron deficiency in rheumatoid arthritis patients (Suominen et al 2000, Zoli et al 1994, Nielsen et al 1994, Punnonen et al 2000). Other studies have not found any consistent deviations in sTfR or sTfR-ferritin ratio among patients with different chronic disorders and anemia (Junca et al 1998,

A slight decrease in sTfR and sTfR:ferritin has been reported in conditions with iron overload (Looker et al 1999, Khumalo et al 1998) but conflicting results have been obtained (Baynes 1994). Raised sTfR in Polycythemia vera and secondary polycythemia is an indicator of iron deficiency (Manteiga et al 1998).

sTfR is higher in heterozygous B-thalassemia patients than in healthy controls and does not differ significantly from sTfR in B-thalassemia concomitant with iron deficiency (Gimferrer et al 1997, Dimitriou et al 2000, Bianco et al 2000). In thalassemia intermedia no consistent patterns have been found (Camaschella et al 1996, Dore et al 1996). In sickle cell anemia sTfR correlates with the degree of erythropoietic expansion, i.e. hypersplenism (Singhal et al 1993).

sTfR ASSAYS

The sTfR assay has gone all the way from labour intensive RIA-methods to fully automated methods implemented on immunoassay analysers common in the clinical laboratory (Vernet et al 2000, Punnonen et al 2000, Suominen et al 1999, Hikawa et al 1996). The intra and interassay precision are good, about 5% coefficient of variation. The linear measuring interval covers the clinically important concentrations. But standardisation is lacking and there is a bias between all different assays (Cotton et al 2000). No internationally accepted calibrator exists (Skikne 1998).

Where sophisticated instrumentation is not at hand, a technique for the measurement of the transferrin receptor/ferritin ratio on plasma spotted and dried on filter paper may be suitable for the identification of moderate to severe iron deficiency anemia (Cook et al 1998, Flowers et al 1999).

PERSPECTIVES

Transferrin receptor concentrations in serum increase with tissue iron deficiency and elevated erythropoiesis. Thus we now have an additional useful marker in addition to old and newer ones, for example reticulocyte maturation index and reticulocyte hemoglobin concentration, in the clinical work up of patients with suspected iron deficiency or erythroid hyperproliferative disorders. A major obstacle

is the lack of standardization between different assay systems. The first step should be to agree upon an international calibrator followed by the establishment of appropriate reference intervals. Current assays have good precision, require only very small sample volumes and are fully automated and so have made it possible to introduce sTfR as a valuable diagnostic tool among the more conventional ones.

As sTfR does not increase in anemia of chronic disease per se, sTfR will help in the evaluation of anemic patients with normal or elevated serum ferritin values. sTfR also promises to be a marker of early functional iron deficiency, commonly seen in pregnant women, premenopausal women, adolescents and blood donors, among others. sTfR measurements have a low total variation, biological and analytical, and hence the clinical value of serial measurements to assess the effect of treatment in the anemic or hyperproliferative patient should be investigated.

Ahluwalia N, Skikne BS, Savin V, Chonko A. Markers of masked iron deficiency and effectiveness of EPO therapy in chronic renal failure. *Am J Kidney Dis.* 1997 Oct;30(4):532-41.

REFERENCES

1. Akesson A, Bjellerup P, Berglund M, Bremme K, Vahter M. Serum transferrin receptor: a specific marker of iron deficiency in pregnancy. *Am J Clin Nutr.* 1998 Dec; 68(6): 1241-6.
2. Allen J, Backstrom KR, Cooper JA, Cooper MC, Detwiler TC, Essex DW, Fritz RP, Means RT Jr, Meier PB, Pearlman SR, Roitman-Johnson B, Seligman PA. Measurement of soluble transferrin receptor in serum of healthy adults. *Clin Chem.* 1998 Jan;44(1):35-9.
3. Anttila R, Cook JD, Siimes MA. Body iron stores decrease in boys during pubertal development: the transferrin receptor-ferritin ratio as an indicator of iron status. *Pediatr Res.* 1997 Feb;41(2):224-8.
4. Baynes RD, Shih YJ, Hudson BG, Cook JD. Production of the serum form of the transferrin receptor by a cell membrane-associated serine protease. *Proc Soc Exp Biol Med.* 1993 Oct;204(1):65-9.
5. Baynes RD, Cook JD, Bothwell TH, Friedman BM, Meyer TE. Serum transferrin receptor in hereditary hemochromatosis and African siderosis. *Am J Hematol.* 1994 Apr; 45(4): 288-92.
6. Beguin Y, Huebers HA, Josephson B, Finch CA. Transferrin receptors in rat plasma. *Proc Natl Acad Sci U S A.* 1988 Jan;85(2):637-40.
7. Beguin Y, Clemons GK, Pootrakul P, Fillet G. Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. *Blood.* 1993 Feb 15; 81(4): 1067-76.
8. Beguin Y, Loo M, R'Zik S, Sautois B, Lejeune F, Rorive G, Fillet G. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. *Blood.* 1993 Oct 1;82(7):2010-6.
9. Beguin Y, Loo M, R'Zik S, Sautois B, Lejeune F, Rorive G, Fillet G. Quantitative assessment of erythropoiesis in haemodialysis patients demonstrates gradual expansion of erythroblasts during constant treatment with recombinant

- human erythropoietin. *Br J Haematol.* 1995 Jan;89(1):17-23.
10. Bianco I, Mastropietro F, D'Asero C, Graziani B, Piergrossi P, Mezzabotta M, Modiano G. Serum levels of erythropoietin and soluble transferrin receptor in the course of pregnancy in non beta thalassemic and beta thalassemic women. *Haematologica.* 2000 Sep;85(9):902-7.
 11. Birkeland KI, Stray-Gundersen J, Hemmersbach P, Hallen J, Haug E, Bahr R. Effect of rhEPO administration on serum levels of sTfR and cycling performance. *Med Sci Sports Exerc.* 2000 Jul;32(7):1238-43.
 12. Boulton F, Collis D, Inskip H, Paes H, Garlick M. A study of the iron and HFE status of blood donors, including a group who failed the initial screen for anaemia. *Br J Haematol.* 2000 Feb;108(2):434-9.
 13. Bzdega T, Turi T, Wroblewska B, She D, Chung HS, Kim H, Neale JH. Molecular cloning of a peptidase against N-acetylaspartylglutamate from a rat hippocampal cDNA library. *J Neurochem.* 1997 Dec;69(6):2270-7.
 14. Camaschella C, Gonella S, Calabrese R, Vischia F, Roetto A, Graziadei G, Mazza U, Cappellini MD. Serum erythropoietin and circulating transferrin receptor in thalassemia intermedia patients with heterogeneous genotypes. *Haematologica.* 1996 Sep-Oct;81(5):397-403.
 15. Camaschella C, Roetto A, Cali A, De Gobbi M, Garozzo G, Carella M, Majorano N, Totaro A, Gasparini P: The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. *Nature Genet.* 2000 25: 14-15.
 16. Carriaga MT, Skikne BS, Finley B, Cutler B, Cook JD. Serum transferrin receptor for the detection of iron deficiency in pregnancy. *Am J Clin Nutr.* 1991 Dec;54(6):1077-81.
 17. Chitambar CR, Massey EJ, Seligman PA. Regulation of transferrin receptor expression on human leukemic cells during proliferation and induction of differentiation. Effects of gallium and dimethylsulfoxide. *J Clin Invest.* 1983 Oct;72(4):1314-25.
 18. Choi JW, Pai SH, Im MW, Kim SK. Change in transferrin receptor concentrations with age. *Clin Chem.* 1999 Sep;45(9):1562-3.
 19. Choi JW, Im MW, Pai SH. Serum transferrin receptor concentrations during normal pregnancy. *Clin Chem.* 2000 May;46(5):725-7.
 20. Choi JW, Kim CS, Pai SH. Erythropoietic activity and soluble transferrin receptor level in neonates and maternal blood. *Acta Paediatr.* 2000 Jun;89(6):675-9.
 21. Choi JW, Pai SH. Change in erythropoiesis with gestational age during pregnancy. *Ann Hematol.* 2001 Jan;80(1):26-31.
 22. Chua E, Clague JE, Sharma AK, Horan MA, Lombard M. Serum transferrin receptor assay in iron deficiency anaemia and anaemia of chronic disease in the elderly. *QJM.* 1999 Oct;92(10):587-94.
 23. Collawn JF, Stangel M, Kuhn LA, Esekogwu V, Jing SQ, Trowbridge IS, Tainer JA. Transferrin receptor internalization sequence YXRF implicates a tight turn as the structural recognition motif for endocytosis. *Cell.* 1990 Nov 30; 63(5): 1061-72.
 24. Collawn JF, Lai A, Domingo D, Fitch M, Hatton S, Trowbridge IS. YTRF is the conserved internalization signal of the transferrin receptor, and a second YTRF signal at position 31-34 enhances endocytosis. *J Biol Chem.* 1993 Oct 15;268(29):21686-92.
 25. Cook JD, Flowers CH, Skikne BS. An assessment of dried blood-spot technology for identifying iron deficiency. *Blood.* 1998 Sep 1;92(5):1807-13.
 26. Cooper MJ, Zlotkin SH. Day-to-day variation of transferrin receptor and ferritin in healthy men and women. *Am J Clin Nutr.* 1996 Nov;64(5):738-42.
 27. Cotton F, Thiry P, Boeynaems J. Measurement of soluble transferrin receptor by immunoturbidimetry and immunonephelometry. *Clin Biochem.* 2000 Jun;33(4):263-7.
 28. Daschner M, Mehls O, Schaefer F. Soluble transferrin receptor is correlated with erythropoietin sensitivity in dialysis patients. *Clin Nephrol.* 1999 Oct;52(4):246-52.
 29. Dimitriou H, Stiakaki E, Markaki EA, Bolonaki I, Giannakopoulou C, Kalmanti M. Soluble transferrin receptor levels and soluble transferrin receptor/log ferritin index in the evaluation of erythropoietic status in childhood infections and malignancy. *Acta Paediatr.* 2000 Oct;89(10):1169-73.
 30. Dore F, Bonfigli S, Gaviano E, Pardini S, Longinotti M. Serum transferrin receptor levels in patients with thalassemia intermedia during rHuEPO administration. *Haematologica.* 1996 Jan-Feb;81(1):37-9.
 31. Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, Tsuchihashi Z, Sigal E, Bjorkman PJ, Schatzman RC: The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci USA* 1998, 95: 1472-1477.
 32. Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Dominguez-Cabrera C, Hortal-Casc n L, Perez-Borges P, Vega-Diaz N, Saavedra-Santana P, Palop-Cubillo L. Diagnosis of iron deficiency in chronic renal failure. *Am J Kidney Dis.* 1999 Sep;34(3):508-13.
 33. Fleming RE, Migas M C, Holden CC, Waheed A, Britton RS, Tomatsu S, Bacon BR, Sly WS: Transferrin receptor 2: continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis. *Proc. Nat. Acad. Sci.* 2000, 97: 2214-2219.
 34. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. *J Lab Clin Med.* 1989 Oct;114(4):368-77.
 35. Flowers CH, Cook JD. Dried plasma spot measurements of ferritin and transferrin receptor for assessing iron status. *Clin Chem.* 1999 Oct;45(10):1826-32.
 36. Gatter KC, Brown G, Trowbridge IS, Woolston RE, Mason DY. Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J Clin Pathol.* 1983 May;36(5):539-45.
 37. Gimferrer E, Ubeda J, Royo MT, Marigo GJ, Marco N, Fernandez N, Oliver A, Padros R, Gich I. Serum transferrin receptor levels in different stages of iron deficiency. *Blood.* 1997 Aug 1;90(3):1332-4.
 38. Gimferrer F, Ubeda J, Remacha AF. Serum transferrin receptor levels are "physiologically" high in heterozygous beta-thalassemia. *Haematologica.* 1997 Nov-Dec; 82(6): 728-9.
 39. Gross CN, Irrinki A, Feder JN, Enns CA: Co-trafficking of HFE, a nonclassical major histocompatibility complex class I protein, with the transferrin receptor implies a role in intracellular iron regulation. *J Biol Chem* 1998, 273: 22068-22074.
 40. Hikawa A, Nomata Y, Suzuki T, Ozasa H, Yamada O. Soluble transferrin receptor-transferrin complex in serum: measurement by latex agglutination nephelometric immunoassay. *Clin Chim Acta.* 1996 Oct 29;254(2):159-72.
 41. Hou CC, Wu SC, Wu SC, Yang WC, Huang TP, Ng YY. Serum transferrin receptor concentration is not indicative of erythropoietic activity in chronic hemodialysis patients with poor response to recombinant human erythropoietin. *Zhonghua Yi Xue Za Zhi (Taipei).* 1998 Aug;61(8):456-62.
 42. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood.* 1990 Jan 1;75(1):102-7.
 43. Iacopetta BJ, Morgan EH, Yeoh GC. Transferrin receptors and iron uptake during erythroid cell development. *Biochim Biophys Acta.* 1982 May 7;687(2):204-10.

44. Iacopetta BJ, Rothenberger S, Kuhn LC. A role for the cytoplasmic domain in transferrin receptor sorting and coated pit formation during endocytosis. *Cell*. 1988 Aug 12;54(4):485-9.
45. Jing SQ, Trowbridge IS. Identification of the intermolecular disulfide bonds of the human transferrin receptor and its lipid-attachment site. *EMBO J*. 1987 Feb;6(2):327-31.
46. Junca J, Fernandez-Aviles F, Oriol A, Navarro JT, Milla F, Sancho JM, Feliu E. The usefulness of the serum transferrin receptor in detecting iron deficiency in the anemia of chronic disorders. *Haematologica*. 1998 Aug;83(8):676-80.
47. Kawabata H, Yang R, Hiramata T, Vuong P T, Kawano S, Gombart A F, Koeffler H P. Molecular cloning of transferrin receptor 2: a new member of the transferrin receptor-like family. *J Biol Chem*. 1999;274:20826-20832.
48. Khumalo H, Gomo ZA, Moyo VM, Gordeuk VR, Saungweme T, Rouault TA, Gangaidzo IT. Serum transferrin receptors are decreased in the presence of iron overload. *Clin Chem*. 1998 Jan;44(1):40-4.
49. Kohgo Y, Nishisato T, Kondo H, Tsushima N, Niitsu Y, Urushizaki I. Circulating transferrin receptor in human serum. *Br J Haematol*. 1986 Oct;64(2):277-81.
50. Lawrence CM, Ray S, Babyonyshev M, Galluser R, Borhani DW, Harrison SC. Crystal structure of the ectodomain of human transferrin receptor. *Science*. 1999 Oct 22;286(5440):779-82.
51. Loh TT, Higuchi DA, van Bockxmeer FM, Smith CH, Brown EB. Transferrin receptors on the human placental microvillous membrane. *J Clin Invest*. 1980 May; 65(5): 1182-91.
52. Lonnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. *Acta Paediatr*. 1994 Apr;83(4):367-73.
53. Looker AC, Loyevsky M, Gordeuk VR. Increased serum transferrin saturation is associated with lower serum transferrin receptor concentration. *Clin Chem*. 1999 Dec;45(12):2191-9.
54. Lorenzo JD, Rodriguez MM, Martin SS, Romo JM. Assessment of erythropoiesis activity during hemodialysis therapy by soluble transferrin receptor levels and ferrokinetic measurements. *Am J Kidney Dis*. 2001 Mar;37(3):550-6.
55. Manteiga R, Remacha AF, Sarda MP, Ubeda J. Serum transferrin receptor in polycythemia. *Haematologica*. 1998 Oct;83(10):958-9.
56. McClelland A, Kuhn LC, Ruddle FH. The human transferrin receptor gene: genomic organization, and the complete primary structure of the receptor deduced from a cDNA sequence. *Cell*. 1984 Dec;39(2 Pt 1):267-74.
57. Nielsen OJ, Andersen LS, Hansen NE, Hansen TM. Serum transferrin receptor levels in anaemic patients with rheumatoid arthritis. *Scand J Clin Lab Invest*. 1994 Feb;54(1):75-82.
58. North M, Dallalio G, Donath AS, Melink R, Means RT Jr. Serum transferrin receptor levels in patients undergoing evaluation of iron stores: correlation with other parameters and observed versus predicted results. *Clin Lab Haematol*. 1997 Jun;19(2):93-7.
59. Omary MB, Trowbridge IS. Biosynthesis of the human transferrin receptor in cultured cells. *J Biol Chem*. 1981 Dec 25;256(24):12888-92.
60. Pan BT, Blostein R, Johnstone RM. Loss of the transferrin receptor during the maturation of sheep reticulocytes in vitro. An immunological approach. *Biochem J*. 1983 Jan 15;210(1):37-47.
61. Parisotto R, Gore CJ, Emslie KR, Ashenden MJ, Brugnara C, Howe C, Martin DT, Trout GJ, Hahn AG. A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. *Haematologica*. 2000 Jun;85(6):564-72.
62. Punnonen K, Irjala K, Rajamaki A. Iron-deficiency anemia is associated with high concentrations of transferrin receptor in serum. *Clin Chem*. 1994 May;40(5):774-6.
63. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*. 1997 Feb 1;89(3):1052-7.
64. Punnonen K, Rajamaki A. Evaluation of iron status of Finnish blood donors using serum transferrin receptor. *Transfus Med*. 1999 Jun;9(2):131-4.
65. Punnonen K, Kaipainen-Seppanen O, Riittinen L, Tuomisto T, Hongisto T, Penttila L. Evaluation of iron status in anemic patients with rheumatoid arthritis using an automated immunoturbidimetric assay for transferrin receptor. *Clin Chem Lab Med*. 2000 Dec;38(12):1297-300.
66. Rothenberger S, Iacopetta BJ, Kuhn LC. Endocytosis of the transferrin receptor requires the cytoplasmic domain but not its phosphorylation site. *Cell*. 1987 May 8;49(3):423-31.
67. Roy CN, Carlson EJ, Anderson EL, Basava A, Starnes SM, Feder JN, Enns CA. Interactions of the ectodomain of HFE with the transferrin receptor are critical for iron homeostasis in cells. *FEBS Lett*. 2000 Nov 10;484(3):271-4.
68. Rusia U, Flowers C, Madan N, Agarwal N, Sood SK, Sikka M. Serum transferrin receptors in detection of iron deficiency in pregnancy. *Ann Hematol*. 1999 Aug;78(8):358-63.
69. Rutledge EA, Enns CA. Cleavage of the transferrin receptor is influenced by the composition of the O-linked carbohydrate at position 104. *J Cell Physiol*. 1996 Aug; 168(2):284-93.
70. Salter-Cid L, Brunmark A, Peterson PA, Yang Y. The major histocompatibility complex-encoded class I-like HFE abrogates endocytosis of transferrin receptor by inducing receptor phosphorylation. *Genes Immun*. 2000 Oct; 1(7): 409-17.
71. Schneider C, Owen MJ, Banville D, Williams JG. Primary structure of human transferrin receptor deduced from the mRNA sequence. *Nature*. 1984 Oct 18-24;311(5987):675-8.
72. Shih YJ, Baynes RD, Hudson BG, Flowers CH, Skikne BS, Cook JD. Serum transferrin receptor is a truncated form of tissue receptor. *J Biol Chem*. 1990 Nov 5;265(31):19077-81.
73. Singhal A, Cook JD, Skikne BS, Thomas P, Serjeant B, Serjeant G. The clinical significance of serum transferrin receptor levels in sickle cell disease. *Br J Haematol*. 1993 Jun;84(2):301-4.
74. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*. 1990 May 1;75(9):1870-6.
75. Skikne BS. Circulating transferrin receptor assay—coming of age. *Clin Chem*. 1998 Jan;44(1):7-9.
76. Suominen P, Punnonen K, Rajamaki A, Irjala K. Evaluation of new immunoenzymometric assay for measuring soluble transferrin receptor to detect iron deficiency in anemic patients. *Clin Chem*. 1997 Sep;43(9):1641-6.
77. Suominen P, Punnonen K, Rajamaki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood*. 1998 Oct 15;92(8):2934-9.
78. Suominen P, Punnonen K, Rajamaki A, Majuri R, Hanninen V, Irjala K. Automated immunoturbidimetric method for measuring serum transferrin receptor. *Clin Chem*. 1999 Aug;45(8 Pt 1):1302-5.
79. Suominen P, Mottonen T, Rajamaki A, Irjala K. Single values of serum transferrin receptor and transferrin receptor ferritin index can be used to detect true and functional iron deficiency in rheumatoid arthritis patients with anemia. *Arth-*

- ritis Rheum. 2000 May;43(5):1016-20.
80. Suominen P, Virtanen A, Lehtonen-Veromaa M, Heinonen OJ, Salmi TT, Alanen M, Mottonen T, Rajamaki A, Irtala K. Regression-based reference limits for serum transferrin receptor in children 6 months to 16 years of age. *Clin Chem*. 2001 May;47(5):935-7.
 81. Vernet M, Doyen C. Assessment of iron status with a new fully automated assay for transferrin receptor in human serum. *Clin Chem Lab Med*. 2000 May;38(5):437-42.
 82. Virtanen MA, Viinikka LU, Virtanen MK, Svahn JC, Anttila RM, Krusius T, Cook JD, Axelsson IE, Raiha NC, Siimes MA. Higher concentrations of serum transferrin receptor in children than in adults. *Am J Clin Nutr*. 1999 Feb;69(2):256-60.
 83. Williams AM, Enns CA. A mutated transferrin receptor lacking asparagine-linked glycosylation sites shows reduced functionality and an association with binding immunoglobulin protein. *J Biol Chem*. 1991 Sep 15;266(26):17648-54.
 84. Yeung GS, Zlotkin SH. Percentile estimates for transferrin receptor in normal infants 9-15 mo of age. *Am J Clin Nutr*. 1997 Aug;66(2):342-6.
 85. Zhu YI, Haas JD. Response of serum transferrin receptor to iron supplementation in iron-depleted, nonanemic women. *Am J Clin Nutr* 1998 Feb;67(2):271-5.
 86. Zoli A, Altomonte L, Mirone L, Magaro M, Ricerca BM, Storti S, Candido A, Bizzi M. Serum transferrin receptors in rheumatoid arthritis. *Ann Rheum Dis*. 1994 Oct; 53(10):699-701.